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TOTAL SOIL NITROGEN ANALYSIS USING MICRO-KJELDAHL DIGESTION AND PORTABLE DISTILLATION EQUIPMENT[1]

by

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ABSTRACT

Equipment modifications and methodology alternatives for determining total nitrogen in soils using a micro-Kjeldahl digestion apparatus were investigated. Results showed that using an air shield over the flask bulbs and a standard copper catalyst-salt mixture should give satisfactory recovery in routine analyses with a running time and sample size similar to that used with macro-Kjeldahl racks. Recovery of several standards was high (96-99 percent), but for recovery of highly refractory compounds, methodology adjustments will be required.

Keywords: Soil analysis, nitrogen, methodology.

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[1]Use of trade, firm, or corporation names is for the information and convenience of the reader and does not constitute an official endorsement or approval by the U.S. Department of Agriculture.

INTRODUCTION

The surge of research in recent decades has created crowded working conditions in many laboratories. Space shortages have encouraged the researcher to use laboratory space more efficiently by employing newer automated instrument methods or smaller versions of larger equipment models when they will do the job required. The latter was my approach to soil nitrogen determinations.

Micro-Kjeldahl equipment is commonly used for plant analyses, and satisfactory results have been obtained with small soil samples (0.2-1.0 g.) ground finer than 80 mesh (Bremner 1960). However, it seemed more desirable to attempt analyses of soil samples similar in size to macro-Kjeldahl samples in order to avoid the grinding, which is required for small sample size, and yet retain reproducibility. In an effort to meet these objectives, micro-Kjeldahl digestion apparatus (Labconco) and portable dual-place distillation equipment (Precision Scientific) were purchased. Both kinds of equipment can be used on the laboratory bench or in hoods without plumbing or electrical modifications.

For methodology consistent with analysis objectives, a number of possible alternatives were considered. Hundreds of variations of the Kjeldahl procedure have been used for N analyses (Kirk 1950). Certainly with the many modifications of digestion time, salts, salt/acid ratios, presoaking, and catalysts, a little experience can lead to considerable frustration in deciding what the proper set of alternatives should be. The best discussions on these topics appear to be those by Kirk (1950) and Bremner (1965b).

The author's early experience resulted in excessive digestion times with slow clearing conditions using the micro-Kjeldahl units; inadequate heating rate for comparatively large samples may have been a reason. The manufacturer's standard heaters (200-watt) were being used with 100-milliliter semi-micro-Kjeldahl flasks, and the digestion units were being subjected to a considerable draft in the hood where they were placed (hood rating 950 cubic feet per minute). An "air shield" was fashioned from galvanized sheet metal to reduce cooling from drafts and surrounding air and to improve heating efficiency. The shield covered the bulb portions but not the necks of the digestion flasks, thus affording lower neck temperatures and refluxing of the sulfuric acid fumes during digestion (fig. 1).

With this equipment modification, an experiment was performed to compare the use of the air shield with other digestion alternatives in an effort to develop a satisfactory method of determining total nitrogen in macro soil samples with the micro-Kjeldahl digestors and portable distillation units.

Figure 1.--*End oblique view of micro-Kjeldahl digestion unit with air shield attached by piano hinge which affords raising and lowering during digestion without interference with controls. The shield can be easily pivoted to check charring, clearing progress, etc.*

METHODS AND MATERIALS

Four alternatives of the digestion phase were considered in conjunction with the same distillation and titration phases of the analyses in a four-treatment randomized block design (a 2 by 2 factorial could have been used but different orthogonal comparisons were desired). Digestion was performed with and without the air shield both in the presence and in the absence of selenium catalyst. A total of 24 soil samples were analyzed in duplicate by each of the four alternative digestion procedures. Samples were reference collections made from upland forest and grassland soils occurring on the Starkey Experimental Forest and Range, 30 miles south of La Grande in Union County, Oregon (Geist and Strickler 1970).

Much of the general procedure which applied to all samples was derived from Bremner (1965b) and Jackson (1958) as follows:

Duplicate 5-gram samples of soil (less than 2-mm.) were placed in 100-milliliter semi-micro-Kjeldahl flasks, and 10 grams of a salt-catalyst

mixture called Kel Pac 2 (10 g K_2SO_4:0.3 g $CuSO_4.5H_2O$) were added, followed by 30-milliliters concentrated sulfuric acid added so as to wash down the flask neck. The flask was swirled to mix the contents and then placed on the digestion unit. Medium heat was applied for 15-20 minutes and then raised to maximum heat for the duration of digestion. Flasks were rotated occasionally, and a refluxing action of the fumes was maintained one-third of the way up the flask neck. After clearing (greenish-white), digestion was continued for 1.5 hours to complete the digestion phase. Flasks were allowed to cool, and the contents were then transferred to 800-milliliter Kjeldahl flasks using several portions of distilled water totalling about 250 milliliters. Transfer is necessary to achieve adequate volume of distillate. Flasks were then further cooled, after which a few pieces of mossy zinc and 100 milliliters of 50-percent (weight/volume) sodium hydroxide were added and the solution swirled to mix. The flasks were then quickly attached to the distillation unit, heat applied, and about 150 milliliters of distillate collected in boric acid containing brom cresol green and methyl red indicators. Titration with standard hydrochloric acid was taken to a light pink end point. In the cases where the air shield was employed, it was raised into position over the digestion flasks after frothing subsided (15-20 minutes after heat was raised to maximum) and remained there until completion of digestion. In cases where selenium catalyst was included, 25 milligrams of selenium powder was added with the aforementioned salt-catalyst mixture.

Determinations were also made of the soil organic matter content by a modified Walkley-Black procedure (Jackson 1958) and soil texture by hydrometer technique.

Because of concern for heat supply and digestion temperatures discussed by Bremner (1965b), Jackson (1958), and Lake et al. (1951), temperatures of several digestion mixtures were taken at the end of the digestion period using a thermocouple as described by Lake et al.

RESULTS

General note was made of digestion time because of the expected effects from the treatments involved. In the case where neither the shield nor a selenium catalyst was used, the time for clearing varied from 1.5 to 2.5 hours. The color of the samples changed more gradually during digestion and thus made diagnosis of clearing point difficult. The time for clearing was 1 hour or less and was less variable when either the shield or selenium catalyst, or both, were used during digestion. In no case was there a problem with excess volumes, frothing over, or improper refluxing action using 100-milliliter flasks and 5-gram soil samples.

Results of the various analyses are presented in Table 1 which shows the diversity of soils involved. Mean values for total nitrogen indicated increased recovery over the check for all treatments. Orthogonal comparisons verified these differences statistically ($F = 59.5$ at 0.05 level). There was also a difference in recovery ($F = 5.5$) between shield or selenium treatments used independently as opposed to both together. Mean values indicated a possible interaction between shield and selenium treatments together; however, this was apparently due to the limited quantity of nitrogen which was reached or approached closely when the treatments were applied together or independently.

Table 1.--*Mean values of duplicate analyses performed on experimental soils*

Soil	Organic matter	Clay content	Soil texture[1/]	Total N			
				No shield or selenium	Shield only	Selenium only	Shield and selenium
Percent				*Parts per million*			
1	3.37	23	l	1,568	1,620	1,626	1,633
2	1.78	25	l	1,006	1,059	1,048	1,064
3	1.49	27	l	858	895	894	908
4	6.52	48	c	2,088	2,117	2,120	2,162
5	6.61	41	c	2,181	2,216	2,267	2,315
6	1.82	49	c	807	818	797	796
7	.94	57	c	419	445	431	430
8	.40	35	cl	206	227	219	215
9	1.73	34	cl	712	745	726	743
10	9.08	40	c	3,112	3,157	3,122	3,181
11	4.66	45	c	1,948	2,026	1,980	2,040
12	1.74	39	cl	868	908	894	932
13	6.01	18	l	1,580	1,793	1,791	1,801
14	3.07	22	l	953	1,107	1,038	1,080
15	1.47	29	cl	592	633	654	638
16	3.75	29	cl	1,799	1,878	1,855	1,927
17	2.70	11	sil	765	805	777	782
18	1.29	9	sil	463	473	483	481
19	2.12	10	l	679	666	695	695
20	1.07	9	l	394	410	411	412
21	4.02	27	cl	1,812	1,896	1,951	1,982
22	2.39	31	cl	1,217	1,306	1,280	1,323
23	1.04	43	c	777	789	805	816
24	.56	65	c	338	379	351	369
Mean				1,131	1,182	1,176	1,197

[1/] l = loam, c = clay, cl = clay-loam, sil = silt-loam.

Temperatures of various boiling mixtures at the end of digestion under various combinations of shield, selenium, and soil use are reported in table 2. Flasks covered by the air shield had somewhat higher digestion temperatures than those not covered by the shield. When flasks were placed on a 750-watt heater, all temperatures read the same (358° C.).

Recovery of standard compounds (10-mg. N) indicated sufficient digestion conditions for breakdown and recovery of less resistant materials but insufficient conditions for hydrolysis of refractory compounds such as pyridine (table 3).

Table 2.-- *Temperature of various Kjeldahl digestion mixtures under different digestion conditions 1.5 hours after clearing at 680 mm. Hg. (soil 19)*

Soil content	Salt-catalyst mixture	Sulfuric acid	Selenium powder	Air shield	Temperature
Grams		*Milliliters*	*Milligrams*		*° C.*
0	10	30	0	No	343
5	10	30	0	No	343
0	10	30	0	Yes	358
5	10	30	0	Yes	353
0	10	30	25	No	340
5	10	30	25	No	343
0	10	30	25	Yes	358
5	10	30	25	Yes	350

Table 3.-- *Standard recovery data under digestion conditions using air shield only*

Compound	Recovery
	Percent
Na_2-EDTA[1/]	96
Glutamic acid	97
Alanine	97
Arginine·HC1	99
Pyridine	61

[1/] Disodium (ethylenedinitrilo) tetraacetate dihydrate.

DISCUSSION AND CONCLUSIONS

Use of the air shield or selenium or both shows several advantages over the use of neither in the digestion procedure described. Higher final digestion temperatures, color of the digest, and differences in clearing time all indicated possible higher recovery (verified in table 1). The time saved during digestion is clearly meaningful and results in a digestion time similar to macro-Kjeldahl racks; however, an increase of 45-66 p.p.m. (parts per million) in total N may be inconsequential to those interested only in comparative N content, even though the increases were statistically significant. No time advantage and very little recovery advantage (15-21 p.p.m. N) were gained using both shield and selenium compared with either alone. The need for recovery of these small amounts will depend partly on the intended use of the analysis and one's ability to duplicate the field sample.

In the author's laboratory, routine duplicate analyses for total nitrogen are considered acceptable if they are within a maximum difference of 25 p.p.m. Although the digestion time and recovery levels were about the same for both selenium and the shield, attaching and using the shield is a one-time expense whereas selenium requires measurement and handling in every set of samples. It is emphasized that there were no physical problems involved with using 5-gram soil samples in any of the determinations.

The general effect of selenium found here and by other researchers in the past was a shorter digestion period. There have been reports of losses of nitrogen with selenium; however, it has been recommended that use of low quantities over short digestion periods should avoid such losses (Kirk 1950). If one were to employ higher wattage heaters with a similar shield and selenium, it would be well to check for possible nitrogen losses.

The lower temperatures which existed in unshielded flasks lacking selenium were probably accompanied by slower digestion rates. The net effect was a somewhat lower recovery and a considerably longer clearing time. Addition of selenium apparently counteracted the lower temperature effects on clearing time.

The conditions of digestion proposed by Lake et al. (1951) were designed to accomplish recovery of pyridine and other refractory compounds expected to be present in shale oil or petroleum products. Soils apparently contain very low levels of refractory compounds. Various Kjeldahl methods, modified for recovery of such materials, as well as conventional methods have been shown to give similar analytical recoveries in a variety of soils (Bremner 1960). The organic nitrogen in surface soils exists mainly as bound amino acids and

combined hexosamines, and purine and pyrimidine derivatives make up 1 perce or less of the total soil nitrogen. The chemical nature of the remainder is stil principally unknown, and the recoverability of fixed-N remains in doubt (Bremner 1965a). Bremner (1965b) recommends a 5-hour digestion after clearing for high accuracy. However, he adds that rarely is recovery less than 98 percent of these values with a 2-hour digestion (for most soils there is no difference), and he suggests that a 1-hour digestion is adequate for routine soil analyses.

Considering the above discussion and standard recoveries reported, I concluded that the method using the air shield with the copper catalyst-salt mixture should yield satisfactory recovery of total N in most soils for routine conditions with a minimum of labor involved. Reasonably large sample sizes should be easily accommodated. In materials where refractory compounds are likely to be present in significant concentration, the proposed method would not be satisfactory without attaining higher final digestion temperatures through increasing the salt/acid ratio or using a longer digestion period, or some combination of both (Lake et al. 1951).

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